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The SNMP/CD36 gene family in Diptera, Hymenoptera and Coleoptera: *Drosophila melanogaster*, *D. pseudoobscura*, *Anopheles gambiae*, *Aedes aegypti*, *Apis mellifera*, and *Tribolium castaneum*

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Abstract

Sensory neuron membrane proteins (SNMPs) are membrane bound proteins initially identified in olfactory receptor neurons of Lepidoptera and are thought to play a role in odor detection; SNMPs belong to a larger gene family characterized by the human protein CD36. We have identified 12–14 candidate SNMP/CD36 homologs from each of the genomes of *Drosophila melanogaster*, *D. pseudoobscura*, *Anopheles gambiae* and *Aedes aegypti* (Diptera), eight candidate homologs from *Apis mellifera* (Hymenoptera), and 15 from *Tribolium castaneum* (Coleoptera). Analysis (sequence similarity and intron locations) suggests that the insect SNMP/CD36 genes fall into three major groups. Group 1 includes the previously characterized *D. melanogaster* emp (epithelial membrane protein). Group 2 includes the previously characterized *D. melanogaster croquemort, ninaD, santa maria*, and *peste*. Group 3 genes include the SNMPs, which fall into two subgroups referred to as SNMP1 and SNMP2. *D. melanogaster* SNMP1 (CG7000) shares both significant sequence similarity and five of its six intron insertion sites with the lepidopteran *Bombyx mori* SNMP1. The topological conservation of this gene family within the three major holometabolous lineages indicates that it predates the coleopteran and hymenoptera/dipera/lepidoptera split 300+ million years ago. The current state of knowledge of the characterized insect members of this gene family is discussed.

Keywords: Olfaction; Chemoreception; Evolution; Insect; Pheromone

1. Introduction

Sensory neuron membrane proteins (SNMPs) were identified and characterized as 2-transmembrane domain membrane proteins (519–525 amino acids) of Lepidoptera (moth) olfactory receptor neurons and suggested to play an important but functionally unknown role in odor detection (Rogers et al., 1997, 2001a, b). A Diptera (fly) SNMP homolog, CG7000, has recently been shown to be required for the detection of an aggregation pheromone (Benton et al., 2007). SNMPs belong to a larger family of two-transmembrane domain proteins characterized by the human fatty acid transporter (FAT) CD36 which has a broad range of described roles including cholesterol transport by macrophage cells, cell–cell recognition or cytoadhesion between a variety of cells, and fatty acid recognition in taste receptor cells (e.g. Rasmussen et al., 1998; Calder and Deckelbaum, 2006; Febbraio and Silverstein, 2007; Fukuwatari et al., 1997; Rac et al., 2007). In addition to the SNMPs, several other CD36 homologs have been described in insects (*Drosophila melanogaster*), including epithelial membrane protein (emp), Croquemort, Peste, NinaD and Santa Maria; similar to CD36, these proteins have functions that include cytoadhesion, caratenoid transport, and chemoreception (see Section 4).

This current report characterizes the SNMP/CD36 family of genes/proteins in the genomes of four species of Diptera (the flies *D. melanogaster* and *D. pseudoobscura*

Abbreviations: CD36, cluster determinant 36; Crq, croquemort; Emp, epithelial membrane protein; NinaD, neither inactivation nor after-potential D, Pes, Peste; SCRB, scavenger receptor type B; SNMP, sensory neuron membrane protein; MYA, million years ago.

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and the mosquitoes *Anopheles gambiae* and *Aedes aegypti*), one species of Hymenoptera (the honeybee *Apis mellifera*) and one species of Colepotera (the red flower beetle *Tribolium castaneum*). The *D. melanogaster* genes were previously reported in Rogers et al. (2001b).

The chosen dipteran species represent significant ranges of well established evolutionary distances (Fig. 1): *D. melanogaster* and *D. pseudoobscura* represent the deepest lineage split among the available drosophilid genomes; *An. gambiae* and *Ae. aegypti* represent the deepest lineage split among available mosquito genomes. The coleopteran, hymenopteran and dipteran/lepidopteran lineages represent the three major holometabolous lineages (Grimaldi and Engel, 2005) and thus offer the opportunity to generalize the observed gene patterns across the entire holometabolous group. Collectively, these genomes offer a view of the evolutionary dynamics of the SNMP/CD36 gene family over a variety of time scales ranging from the divergence of the *D. melanogaster* and *D. pseudoobscura* lineages (43–65 MYA, Tamura et al., 2004) to the divergence of the coleopteran and hymenopteran/dipteran/lepidopteran lineages (300+ MYA, Grimaldi and Engel, 2005).

2. Methods

2.1. Identification of genes and gene structures

Amino acid sequences from 13 previously reported *D. melanogaster* SNMP/CD36 homologs (Rogers et al., 2001b) were used in a BLAST-P search of the *D. pseudoobscura*, *An. gambiae, A. mellifera*, and *T. castaneum* genomes using the NCBI website. The *An. gambiae* sequences were subsequently used in a BLAST-P search of the *Ae. aegypti* genome using the NCBI website. Sequences with an *e*-value of less than 0.005 were selected (Karlin and Altschul, 1990), and these were re-blasted to find any additional presumptive homologs within respective species using the same criterion. All sequences contained at least part of the approximately 400 amino acid CD36 motif, identified by Conserved Domain function of the BLAST-P analysis. Most sequences contained the full motif, shorter sequences not containing the full CD36 motif may be incomplete. The full set of sequences identified are listed in Table 1. Gene positions, orientations and intron insertion sites for *D. melanogaster* and *An. gambiae* were determined using the NCBI Genome Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/) and the sequence view link (SV) on the Map Viewer associated with a specific gene. Gene positions and orientations for *D. pseudoobscura* and *Ae. aegypti* were determined from the annotated scaffolds associating with each gene; intron insertion sites were determined by translating the indicated DNA sequence and noting the exon/intron locations.

Two of the *An. gambiae* sequences were combined out of multiple gene entries. EAA07966 (SCRB11) and EAA07986 (SCRB1) were combined in linear fashion; these computer annotated entries were contiguous in the genome, separated by about 70 bp; alignment with other SNMP homologs suggested SCRB11 comprised the 5′ half and SCRB1 comprised the 3′ half of the CD36 motif. Similarly, EAA11087 (SCRB12), EAA11632 (SCRB2), and EAA11629 (SCRB4) were also combined in linear fashion; these computer annotated entries were contiguous in the genome, separated by 2500 bp (SCRB12-SCRB2) and 6000 bp (SCRB2-SCRB4), and alignment with other SNMP homologs suggested SCRB12 comprised the 5′ third, SCRB2 the middle third and SCRB4 the 3′ third of the CD36 motif. Another gene is present within the proposed intron between SCRB2 and SCRB4, but in the opposite orientation of the SCRB segments (XM_315732, ENSANGP00000015904).

One *Ae. aegypti* sequence, referred to here as Aa-SNMP2, was combined from three gene entries: EAT42493, EAT42492, and EAT42490 (5′ to 3′). All three segments are in the same orientation, the first and second separated by 12,062 bp and the second and third by 12,635 bp. Structural analysis in the Blast data suggest

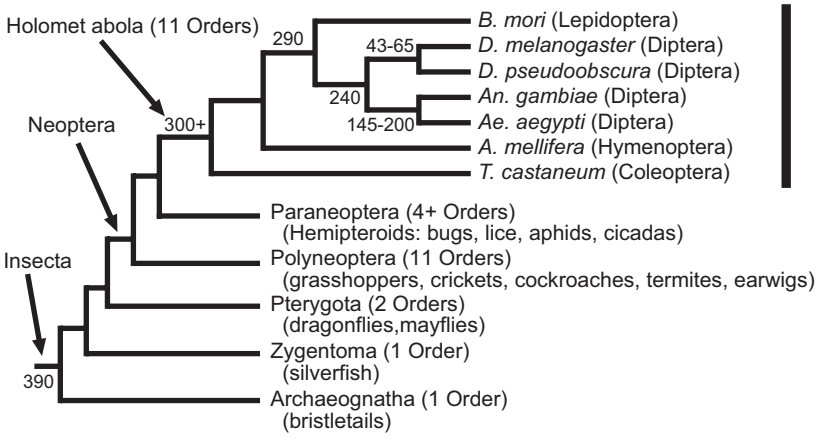

Fig. 1. Phylogenetic relationships of the insect species and groups characterized in this study. Numbers indicate time (MYA) since lineages shared a common ancestor. Organization and times are from Grimaldi and Engel (2005) (see Section 4).

Table 1

	Accession no.	Gene name	No. of amino acids	Group (NJ)	Orthologue
D. melanogaster (Diptera)					
1	CG1887		513	1a	1
2	CG2727	Emp	551	1a	2
3	CG2736		507	1b	3
4	CG3829		597	1a	4
5	CG4280	Crq	491	2a	5
6	CG7000	SNMP	551	3	6 (SNMP1)
7	CG7422	(EU189152)	555	3	7 (SNMP2)
8	CG7227		518	2b	8
9	CG7228	Pes	589	2b	9*
10	CG10345		552	1b	10
11	CG12789	Santa Maria	563	2b	11
12	CG31741		491	2a	12
13	CG31783	NinaD	513	2a	9**
14	CG40006		689	1b	13
D. pseudoobscura (Diptera)					
1	GA10261		556	1b	10
2	GA15107		493	1a	1
3	GA15449		520	1a	2
4	GA15450		500	1b	3
5	GA17715		599	1a	4
6	GA16439		409	2a	12
7	GA16473		504	2a	9**
8	GA18078		489	2a	5
9	GA20018		561	3	6 (SNMP1)
10	GA20195		512	2b	8
11	GA20196		558	2b	9*
12	GA20388		254	3	7 (SNMP2)
An. gambiaea (Diptera)					
1	EAA06468	SCRB10	343	2c	"SCRB10"
2	EAA09639		413	2b	11
3	EAA07765	SCRB5	480	1b	13
4	EAA07966 + EAA07986	SCRB11,1	525	3	6 (SNMP1)
5	EAA09676	SCRB8	507	1a	4
6	EAA09702	SCRB7	342	1b	3
7	EAA09703	SCRB9	522	1a	2
8	EAA10557	SCRB6	391	1b	10
9	EAA11087 + EAA11632 + EAA11629	SCRB12,2,4	476	3	7 (SNMP2)
10	EAA11624	SCRB3	437	1a	1
11	EAA12460	SCRBQ3	459	2a	9*, 9**
12	EAA13815	SCRBQ2	492	2a	5
13	EAA13987	SCRBQ1	461	2a	8
Ae. aegypti (Diptera)					
1	EAT36719		574	1b	13
2	EAT38706		486	2a	5
3	EAT38707		495	2a	8
4	EAT38708		487	2b	9*, 9**
5	EAT39872		394	2c	11
6	EAT40517		481	1a	"SCRB10"
7	EAT42483		530	1a	1
8	EAT42493 + EAT42492 + EAT42490	(EU189151)	542	3	7 (SNMP2)
9	EAT43165		529	3	6 (SNMP1)
10	EAT46038		440	1b	10
11	EAT48768		518	1b	3
12	EAT48768		543	1a	2
13	EAT48770		572	1a	4

Table 1 (*continued*)

	Accession no.	Gene name	No. of amino acids	Group (NJ)	Orthologue
A. mellifera (Hymenoptera)					
1	XP_392321		457	2	
2	XP_394457		535	2	
3	XP_396085		537	1b	10
4	XP_396241		541	1b	13
5	XP_396852		577	1a	1
6	XP_397430		520	3	6 (SNMP1)
7	XP_001120881 + XP_392752		499	1a	2
8	XP_001121085		469	3	3 (SNMP#)
T. castaneum (Colepotera)					
1	XP_966331		515	1a	2
2	XP_968067		541	1a	4
3	XP_968534		568	1b	13
4	XP_969679		529	1b	10
5	XP_969729		384	3	(SNMP#)
6	XP_970008		507	3	(SNMP#)
7	XP_970148		163	?	
8	XP_971582		514	1a	3
9	XP_971917		539	1a	1
10	XP_975231		507	2	
11	XP_975239		351	2	
12	XP_975247		495	2	
13	XP_975606		341	3	(SNMP#)
14	XP_975648		463	2	
(4)	XP_976270	(Same as #4, XP_969679)	514		

Accession numbers of translated genes identified in this study are listed in numerical order along with specific gene names where available, and number of amino acids in the annotated sequence. Multiple accession numbers indicate annotations that were combined in this manuscript to assemble a single contig (see Section 2). Group numbers refer to the Neighbor Joining Tree in Fig. 2 and subsequent figures. Ortholog numbers indicate presumptive orthologs suggested by the Neighbor Joining Tree in Fig. 2 and subsequent figures. "#9*" and #9**" refer to members of two gene clusters in *D. melanogaster* and *D. pseudoobscura* that are suggested to be orthologous with a single gene cluster in *An. gambiae* and *Ae. aegypti* (see Section 3). *T. castaneum* sequences XP_969679, XP_976270 are the same, differing only by an additional 15 N-terminal amino acids in genbank:XP_969679. Because nearly all the *An. gambiae* genes were named, SCRB# (Scavenger Receptor type B), these names are used in some figures to identify orthologs groups.

these entries form the 5′ third, middle third and 3′ third of the CD36 motif, and the combined sequence aligns with other SNMP/CD36 taxa with minimal gapping.

Two *A. mellifera* sequences were tentatively combined: XP_001120881 and XP_392752. Although these were reportedly present in separate scaffolds, XP_001120881 (112 amino acids) contained the 5′ third of the CD36 motif while XP_392752 (387 amino acids) contained the middle and 3′ thirds of the CD36 motif.

The gene of the *Bombyx mori* SNMP1 (SNMP1*Bmor*) was identified using translated amino acid sequence of the reported cDNA (AJ251958) as a Blast query (tblastn) against the *B. mori* genome using the Kaikoblast server (http://kaikoblast.dna.affrc.go.jp/). This genome is

reported in small scaffolds; the entire SNMP1*Bmor* genomic coding sequence was identified as nine exons residing within six scaffolds: 1. BAAB01020456 (exon 1); 2. BAAB01174100 (exon 2); 3. BAAB01036014 (exons 3,4); 4. BAAB01152494 (exon 5); 5. BAAB01132750 (exons 6,7); 6. BAAB01129720 (exons 8,9). Scaffolds 4–6 contained overlapping sequences allowing these to be assembled into a single contig; thus the complete intron sequence was not obtained between e1/e2, e2/e3, and e4/e5. Scaffolds were translated and exons identified manually; all exons were bounded by AG and GT at their respective 3′ and 5′ ends, several showed split codons. SNMP1*Bmor* was aligned with *D. melanogaster* CG7000 using ClustalX, and exon units were marked for each to construct Fig. 6.

2.2. Characterization of genes

Amino acid sequences were used as annotated in the respective genomes, except as noted above, and were aligned with known lepidopteran SNMPs and representative vertebrate CD36 homologs using ClustalX (Thompson et al., 1997; default parameters). This alignment was used to construct the neighbor joining tree shown in Fig. 2 (MEGA3, molecular evolutionary genetics analysis, version 3.1; *p*-distance, gaps handled by pairwise deletion and bootstrap values determined based on 1000 replicates) (Kumar et al., 2004).

A ClustalX alignment of dipteran sequences and the lepidopteran *B. mori* SNMP1 sequence was used to assign character positions to intron insertion sites. These positions are graphically represented in Figs. 3–6. Binary matrix tables were constructed based on the presence or absence of these insertion sites, coded as G or T; these tables were analyzed using MEGA3 software to construct trees characterizing gene relationships based on intron insertion site position (e.g. Fig. 4C).

3. Results

3.1. SNMP/CD36 gene family of diptera

The complete list of sequences identified as presumptive SNMP/CD36 homologs in *D. melanogaster*, *D. pseudoobscura*, *An. gambiae* and *Ae. aegypti* is presented in Table 1. The six previously characterized members from *D. melanogaster* (Emp, Crq, Nina-D, Santa Maria, Peste, CG7000 (SNMP)) range in size from 491 to 589 amino acids (avg. 543). The entire group of translated annotated sequences ranged in length from 254 to 689 amino acids (avg. 496). These size ranges are represented graphically in Fig. 3; presumably many of these lengths will be modified as complete cDNAs are characterized. Of the four genomes assayed, that of *Ae. aegypti* is notably about ten times larger than the other three, due to the large numbers of transposons present within intronic and intergenic regions (Nene et al., 2007); this is reflected in data presented relating to gene size and intergenic distances within gene clusters.

Distance analysis (neighbor joining tree) of the amino acid alignment of these sequences, along with a subset of mammalian (cd36) and lepidopteran (SNMP) homologs, is shown in Fig. 2A. We suggest an organization of the insect genes into three groups (1–3) and several subgroups (1a and b, 2a–c), in part based on bootstrap support of branch groupings in the tree and in part based on gene clustering (see below). In general, genes from each species group together in the tree in a manner suggesting orthologs relationships. The presumptive orthologs dipteran genes of Groups 1 and 3 appear to be more highly conserved than those in Group 2; Group 1 and 2 fly and mosquito genes group together in the same branches while Group 2 fly and mosquito genes segregate.

The chromosomal locations of the SNMP/CD36 homologs of *D. melanogaster* and *An. gambiae* are shown in Fig. 2B. Several of these genes are present in doublet or triplet arrays with no other intervening genes. Assuming the groupings in Fig. 2A represent orthologs genes, the relative positions of these genes is clearly different between *D. melanogaster* and *An. gambiae*, presumably due to the occurrence of translocation events after the lineages of these two species diverged.

An amino acid alignment of all dipteran SNMP/CD36 homologs plus the lepidopteran *B. mori* SNMP1 is represented in Fig. 3, showing only the positions of intron insertion sites (and initial and final amino acid positions) within these aligned sequences. Taxa are arranged in the order of the ClustalX alignment, and thus strongly influenced by sequence similarity. Many intron insertion sites are clearly conserved across this family in all four species. A maximum parsimony tree was constructed using these intron insertion sites as characters (see Section 2); while this analysis grouped many presumptive orthologs pairs, the overall topology of the tree was weakly supported, presumably due to the low number of informative characters, and is therefore not shown. The intron insertion sites are further discussed below in the context of the individual groups.

3.2. Group 1 Dipteran Genes (including *D. melanogaster emp*)

The Group 1 genes of Fig. 2A form six subgroups, all but one of which includes one gene from each of the four dipteran species. The exception is the subgroup including *An. gambiae scrb5* which lacks an orthologs gene in *D. pseudoobscura*; the *D. pseudoobscura* ortholog of *scrb5* is either lost, or was missing from the genome annotation and therefore overlooked. Each subgroup is presumed to include orthologs genes from the respective species. These presumed orthologies are further supported by conservation of intron insertion sites within each subgroup (Fig. 4A). The only Group 1 gene that has

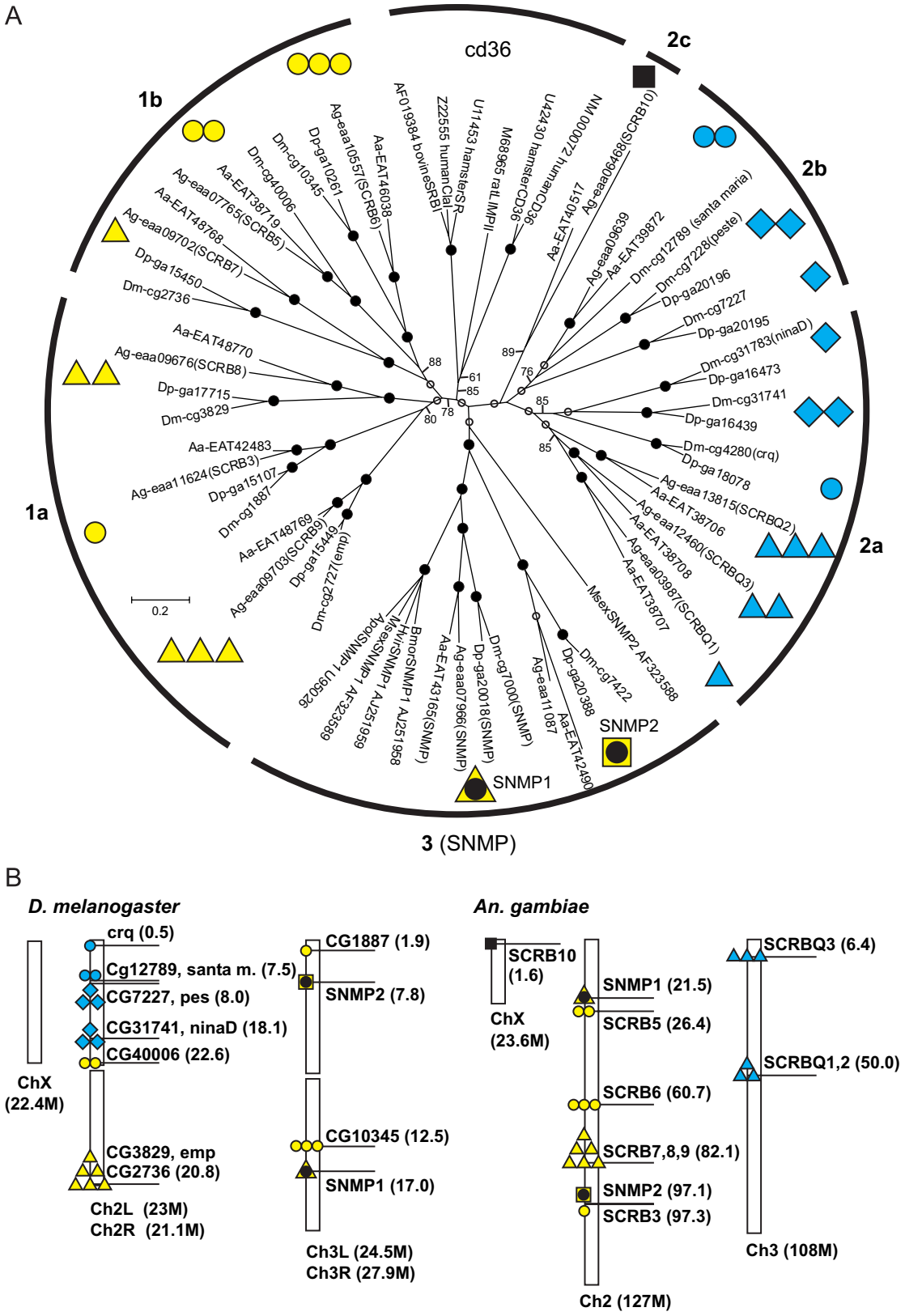

Fig. 2. Sequence comparison of Dipteran, Lepidopteran, and Vertebrate SNMP/CE36 homologs. (A) A Neighbor Joining Tree of SNMP/CD36 homologs listed in Table 1, including the lepidopteran SNMPs and representative vertebrate cd36 homologs of vertebrates. The dipteran genes have been assigned to three groups (1–3) and several subgroups (1a and b, 2a–c), based on bootstrap support and gene clustering. Bootstrap support is based on 1000 replicates, and is indicated either by symbol (closed circles: 95–100%; open circles: 90–94%) or number. (B) Chromosomal locations of *D. melanogaster* and *An. gambiae* SNMP/CD36 homologs. Numbers indicate approximate nucleotide position in megabases; these numbers refer to positions in chromosomal arms for *D. melanogaster*, and to entire chromosomes for *An. gambiae*. The entire size of the respective chromosomal units is indicated below each unit, also in megabases. Colored symbols mark presumed orthologs clusters.

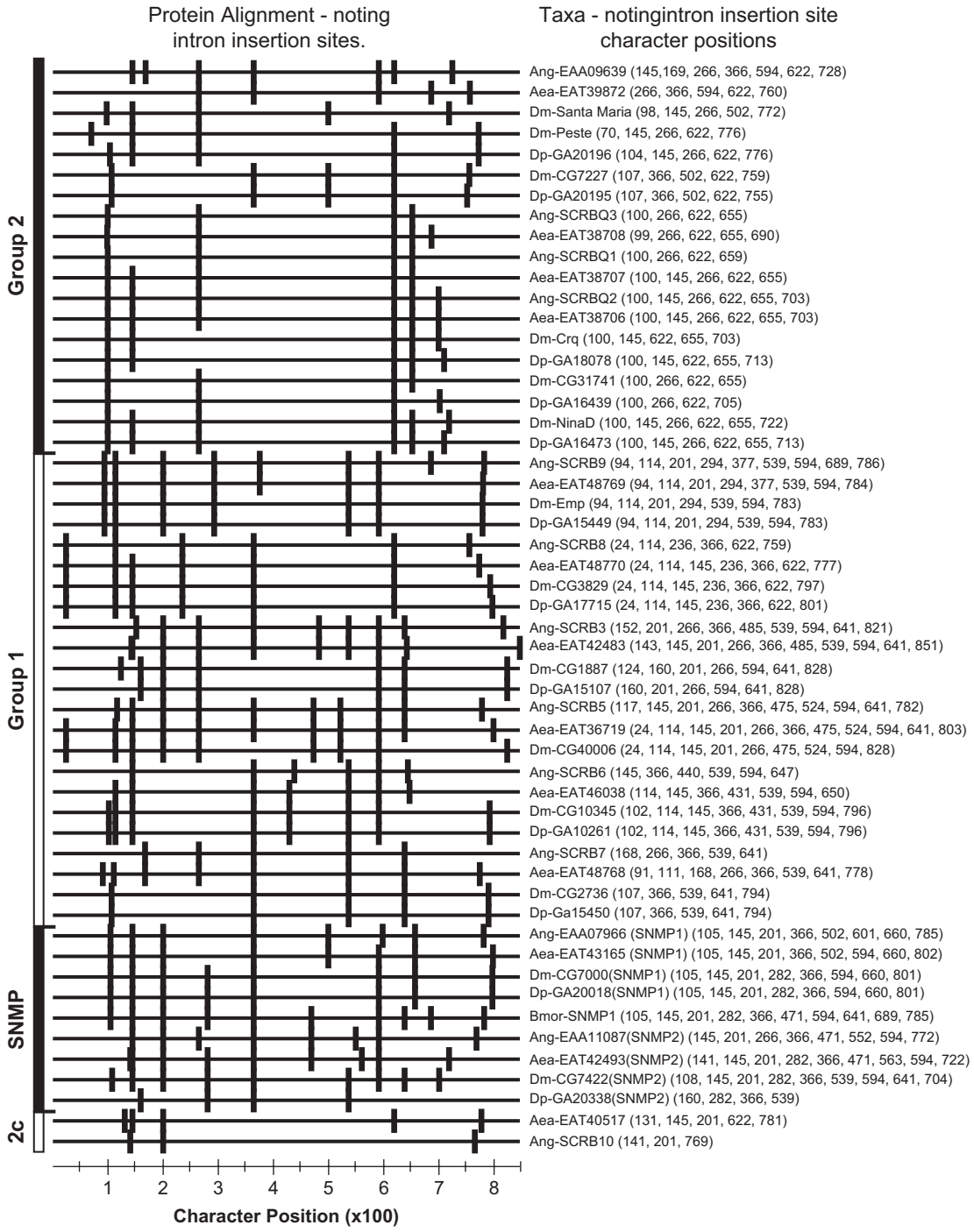

Fig. 3. Comparison of intron insertion sites. Alignment of intron insertion sites for dipteran SNMP/CD36 homologs and *B. mori* SNMP1. The figure is based on a ClustalX alignment of amino acid sequences, exact character positions (taken from text file) are noted next to each taxon name. Dm, Dp, Ang, Aea refer to *D. melanogaster*, *D. pseudoobscura*, *An. gambiae* and *Ae. aegypti*. The order of taxa was taken from the ClustalX output file. The taxon groups defined in Fig. 1 are indicated (2C refers to Group 2C). The far left and far right sites indicate first and last amino acids in the annotated sequences and are not necessarily intron insertion sites; these sites were not included in subsequent analyses of intron insertion sites. Character positions greatly exceed the actual number of amino acids in any given sequence because of gaps present in the alignment.

been characterized is *D. melanogaster emp* (CG2727) (see Section 4).

Genes from three of the Group 1 subgroups reside in clusters in all four species; these subgroups include the *An. gambiae* genes *scrb7*, *scrb8*, and *scrb9*. Fig. 4B represents these clusters, noting the position and orientation of each gene within the cluster as well as the approximate number of base pairs separating each gene. Gene order is consistent with the orthologs relationships suggested by the subgroupings in Figs. 2A and 4A. However, while

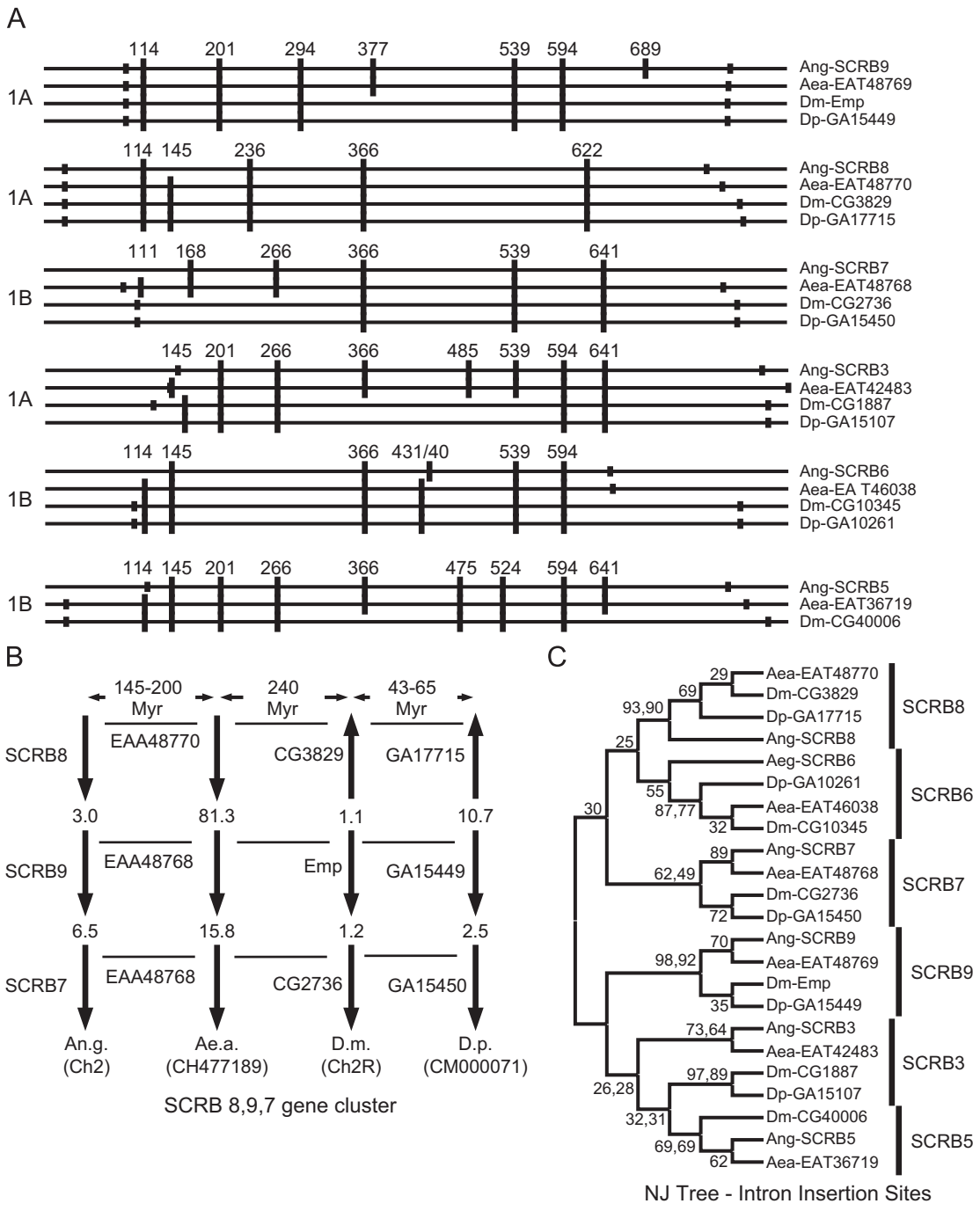

Fig. 4. Dipteran SNMP/CD36 Group 1. (A) Intron insertion site alignment, taken from Fig. 3, but including suggested initial and final amino acid positions (short vertical bars). "1A" and "1B" indicate subgroups from Fig. 2A. (B) The orientation and relationships of the SCRB7,8,9 gene cluster and its orthologs in the four dipteran species. Numbers between arrows indicate the basepair distance between the respective genes in kilobases. Numbers at the top indicate the approximate time period when the respective species lineages diverged (MYA). Numbers at the bottom indicate chromosome or gene scaffold number. (C) A Neighbor Joining Tree showing the relationships of the Group 1 genes, based on Intron Insertion sites as characters. Bootstrap values are shown, based on 1000 replicates; where two values are shown, the second value is the bootstrap support for that branch topology using Maximum Parsimony tree.

the orientation of all three genes is the same in both mosquito species, the presumed fly orthologs of *An. gambiae* SCRB8 are in opposite orientation from their respective mosquito partners (orthologs of *An. gambiae scrb9* and *scrb7*). The presence of this gene cluster in all four species suggests that it arose from gene duplication events prior to the split between mosquito and fly lineages. An independent event presumably occurred reversing the direction of the *scrb8* orthologs, but only in either the mosquito or fly lineage and ancestral to the generation of the respective species. Maximum parsimony and neighbor joining analysis of intron insertion sites

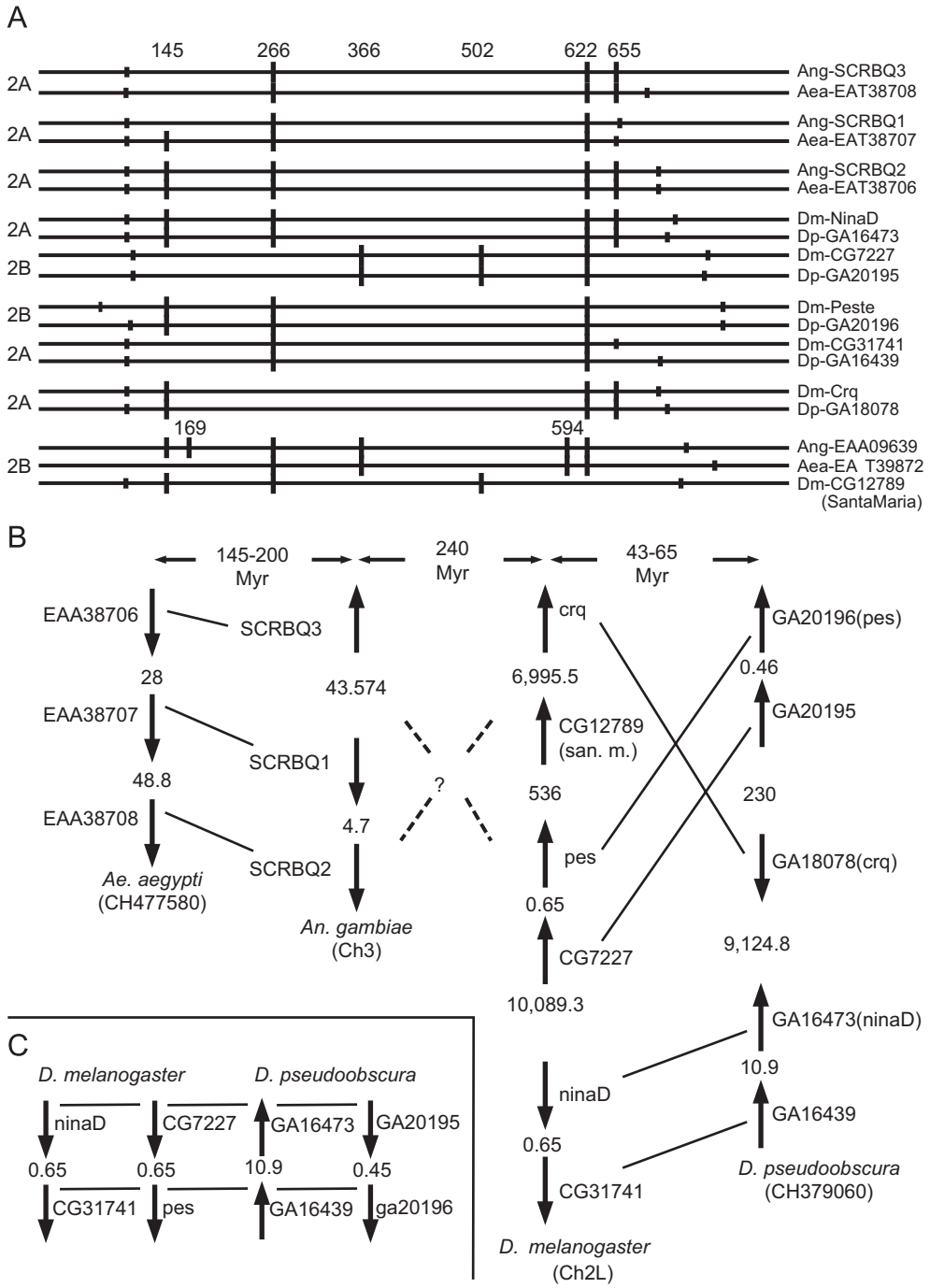

Fig. 5. Dipteran SNMP/CD36 Group 2. (A) Intron insertion site alignment, taken from Fig. 3; initial and final amino acid positions are noted by short vertical bars. "2A", "2B" and "2C" indicate subgroups from Fig. 2A. (B) The orientation and relationships of the SCRBQ1,2,3 gene cluster and its orthologs in the four dipteran species. Numbers between arrows indicate the basepair distance between the respective genes in kilobases. Numbers at the top indicate the approximate time period when the respective species lineages diverged (MYA). Numbers at the bottom indicate chromosome or gene scaffold number. Solid lines suggest orthologs genes; dashed lines and question mark between the mosquito and fly genes indicate uncertainty regarding the exact orthologs relationships between these species. (C) The orientation and relationships of pairs of gene clusters in *D. melanogaste*r and *D. pseudoobscura*, also shown in Fig. 5B. Numbers between arrows indicate the basepair distance between the respective genes in kilobases.

(Fig. 4C) suggests a relationship between the *scrb8* and *scrb6* subgroups, suggesting that the SCRB6 subgroup may have derived from a duplication and translocation of an *scrb8* ancestor. This analysis suggests a similar relationship between the SCRB9, *scrb3*, and *scrb5* subgroups, suggesting that *scrb3* and/or *scrb5* may have derived from duplication and translocation of an *scrb9* ancestor. However, support for these relationships is weak,

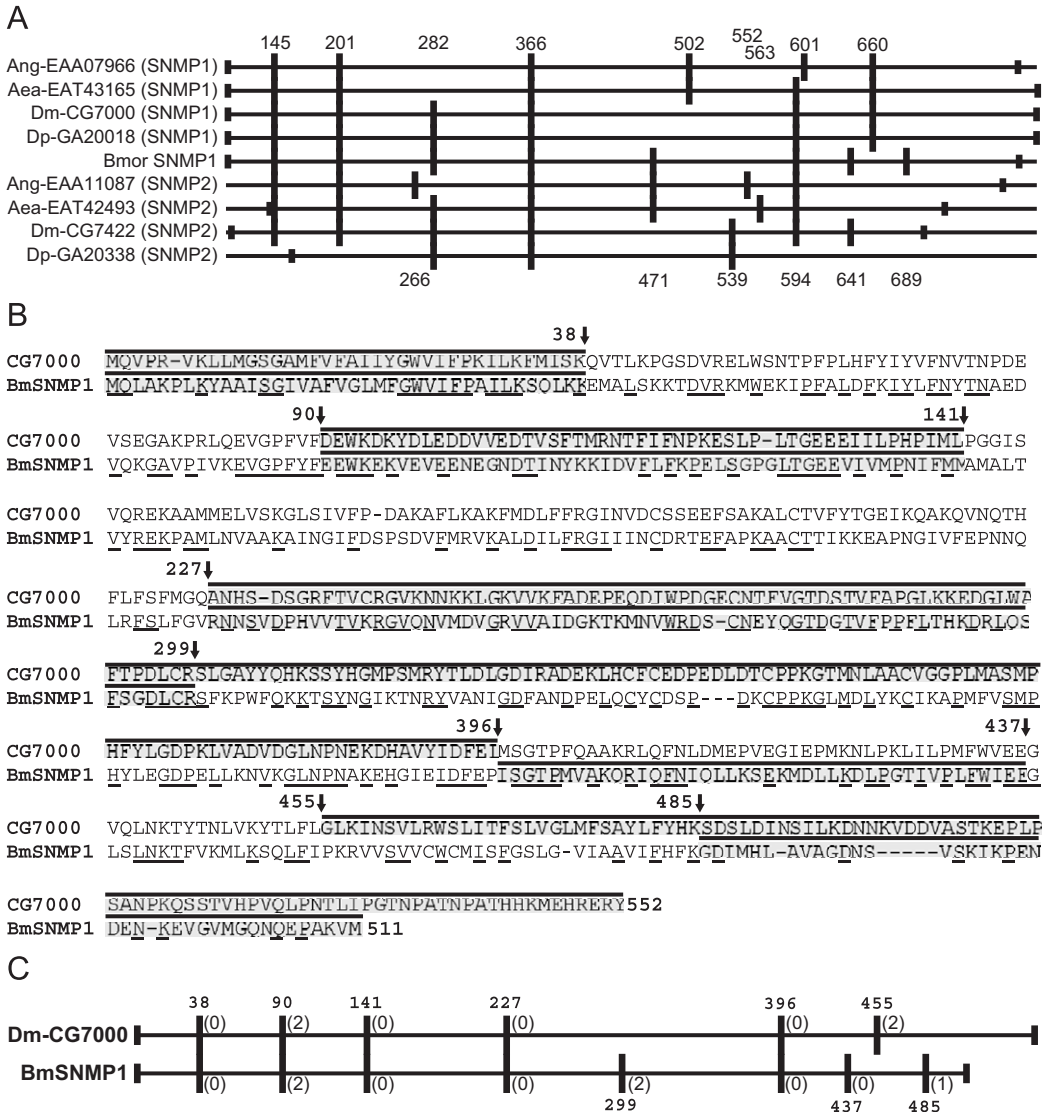

Fig. 6. Dipteran SNMP/CD36 Group 3. (A) Intron insertion site alignment, taken from Fig. 3, but including suggested initial and final amino acid positions (short vertical bars). Insertion site character positions are noted. (B) Alignment of *D. melanogaster* CG7000 and *B. mori* SNMP1. Alternating exon units are noted as shaded or unshaded and overlines; arrows mark intron insertion sites, accompanied by associating character position (amino acid to the left of the insertion site). Broken line below sequence indicates identical amino acids. (C) Alignment of *D. melanogaster* CG7000 and *B. mori* SNMP1 showing only intron insertion sites; character positions are from Fig. 6B. Numbers in parentheses indicate the phase of each intron insertion site.

presumably due to the relatively few informative characters available for analysis.

3.3. Group 2 Dipteran genes (including *D. melanogaster crq, pes, ninaD, santa maria*)

The Group 2 genes of Fig. 2A form multiple subgroups, most of which segregate between fly and mosquito. Two mosquito genes are identified as Group 2c in Fig. 2A; while likely orthologs, these are quite divergent from the other CD36 family members. The majority of Group 2 genes are identified as either 2a or 2b; these genes show conserved patterns of intron insertion sites which in general distinguish them from the other insect CD36 family members (Figs. 3 and 5A). While all of the Group 2a,b genes are located on the same chromosome in each species (Fig. 5B), the relationships and evolutionary history of these genes seem more complicated than those of the Group 1 genes. Several Group 2 genes from *D. melanogaster* have been previously characterized including *croquemort* (*crq*, CG4280), *ninaD* (CG31783), *santa maria* (CG12789), and *peste* (*pes*, CG7228) (see Section 4).

Figs. 5B and C note the relative chromosomal positions of the Group 2a,b genes and suggest their orthologs relationships. In *Ae. aegypti,* three of the Group 2 genes form a cluster with no intervening genes (Fig. 5B). *An. gambiae* has apparent orthologs of the *Ae. aegypti* cluster members, supported in part both by sequence similarity (Fig. 2A) and conserved intron insertion sites (Fig. 5A). Two of the *An. gambiae* genes, *scrbQ1* and *scrbQ2*, are in

tandem array and in the same relative orientation as their *Ae. aegypti* orthologs (eaa38707 and eaa38708); a third *An. gambiae* gene, *scrbQ3*, is located elsewhere on the same chromosome and in opposite orientation than its *Ae. aegypti* ortholog (eaa38706), apparently the result of a translocation event within the *An. gambiae* lineage (Fig. 5B).

D. melanogaster has six Group 2a,b genes; two pairs form tandem arrays (*ninaD*/*cg31741* and *cg7227*/*pes*) and two are isolated (*crq* and *santa maria*). *D. pseudoobscura* has five Group 2a,b genes; two pairs form tandem arrays (*ga16473*/*ga16439* and *ga20195*/*ga20196*) and one is isolated (*ga18078*). The likely orthologs relationships of the *D. melanogaster* and *D. pseudoobscura* genes are indicated in Fig. 5B, supported by sequence similarity (Fig. 2A), conserved intron insertion sites (Fig. 5A) and gene order (Fig. 5B). Our survey of the *D. pseudoobscura* genome did not identify a candidate ortholog of the *D. melanogaster* gene *santa maria* (*cg12789*).

The two tandem arrays in *D. pseudoobscura* and *D. melanogaster* likely derived from an ancestral duplication of one array, as appropriate members of these doublets share sequence similarity (Fig. 2A) and conserved intron insertion sites (Fig. 5A). Curiously, one of the *D. pseudoobscura* duplexes (*ga16473* and *ga16439*) appears in opposite orientation from the other duplexes (Fig. 5C); *ga16473* is situated 3′ of its partner, while its presumed orthologs are all situated 5′ of their partners. One possible explanation is that *ga16473* was repositioned by a translocation event; this would be consistent with the increased gap separating this duplex, as relatively small gaps separate the related duplexes (Fig. 5C).

The specific relationships of the Group 2a,b genes between mosquito and fly are not clear. While it seems plausible that the mosquito and fly clusters share ancestry, the exact relationships are clouded by relatively weak sequence similarity (Fig. 2A) and non-informative (i.e. highly conserved) intron insertion sites. Our analysis does not reveal the exact history between mosquito and fly, beyond supporting the general relationships of these genes.

3.4. Group 3 Dipteran genes (SNMPs)

The Group 3 genes of Fig. 2A include the Lepidoptera SNMPs plus two genes from each of the four dipteran species; the dipteran genes form two distinct sub-groups tentatively named *snmp1* and *snmp2*. A comparison of intron insertion sites indicates multiple conserved sites throughout this group, as well as consistent differences between the dipteran *snmp1*s and *snmp2*s (Fig. 6A). Many of the intron insertion sites are also conserved between Diptera and Lepidoptera; *B. mori snmp1* shares seven intron insertion sites with the dipteran *snmp*s (Fig. 6A), including five with *D. melanogaster snmp1* (CG7000) (Figs. 6B and C). However, most of these conserved sites are also present in other dipteran CD36 homologs (Fig. 3), suggesting none are strongly diagnostic of these Group 3 genes.

3.5. SNMP/CD36 gene family of Hymenoptera (*A. mellifera*) and Coleoptera (*T. castaneum*)

Diptera and Lepidoptera emerged comparatively recently among the insect groups; the available genomes of *A. mellifera* (Hymenoptera) and *T. castaneum* (Coleoptera) allowed us to survey the SNMP/CD36 gene family deeper within the holometabolous insect group and to thus sample each of the major lineages of this group. The complete list of sequences identified as presumptive SNMP/CD36 homologs in *A. mellifera* and *T. castaneum* is presented in Table 1. Eight genes were identified from *A. mellifera*; each contains the full CD36 structural motif with translations ranging in length from 457 to 577 amino acids. Fourteen genes were identified from *T. castaneum*; 10 contain the full CD36 structural motif with translations ranging in length from 463 to 568 amino acids. The remaining four *T. castaneum* genes have translations from 163 to 384 amino acids (see Table 1) and may be incomplete annotations; these sequences contain overlapping regions of the CD36 motif and therefore are presumed to represent unique genes. XP_970148 (163 amino acids) was excluded from subsequent analysis due to its short length.

Distance analysis (neighbor joining tree) of the *A. mellifera* and *T. castaneum* sequences are shown in Fig. 7, along with those represented in Fig. 2. Groups 1–3 are maintained with strong bootstrap support (Fig. 7A). *T. castaneum* has supportable orthologs in each of the Group 1 subgroups (Fig. 7B). *T. castaneum* has a similar number of Group 2 genes as each of the dipteran species, however, the orthologs relationships are less clear than in Group 1, as is also the case for the dipteran genes (Fig. 7C). *T. castaneum* has several Group 3 genes, including two strongly supported SNMP2 orthologs, but no obvious SNMP1 ortholog (Fig. 7D). *A. mellifera* has four Group 1 genes which cluster with orthologs subgroups (Fig. 7B), two Group 2 genes with unclear orthologies (Fig. 7C), and two Group 3 genes, one of which is strongly supported as a SNMP1 ortholog (Fig. 7D). The strong support for Groups 1–3 with the inclusion of the Hymenopteran and Coleopteran genes suggests that this overall organization applies across the holometabolous orders.

4. Discussion

We have identified and characterized the relationships of members of the SNMP/CD36 gene family in six species of insect representing the three major holometabolous lineages. We identified 12–14 genes for each of the dipteran and coleopteran species, but only eight for the hymenopteran *A. mellifera*. We present the gene family in three groups supported by sequence similarity, gene structure and a history of gene duplications. Group 1, which includes the *D. melangaster* gene *emp*, includes six orthologs

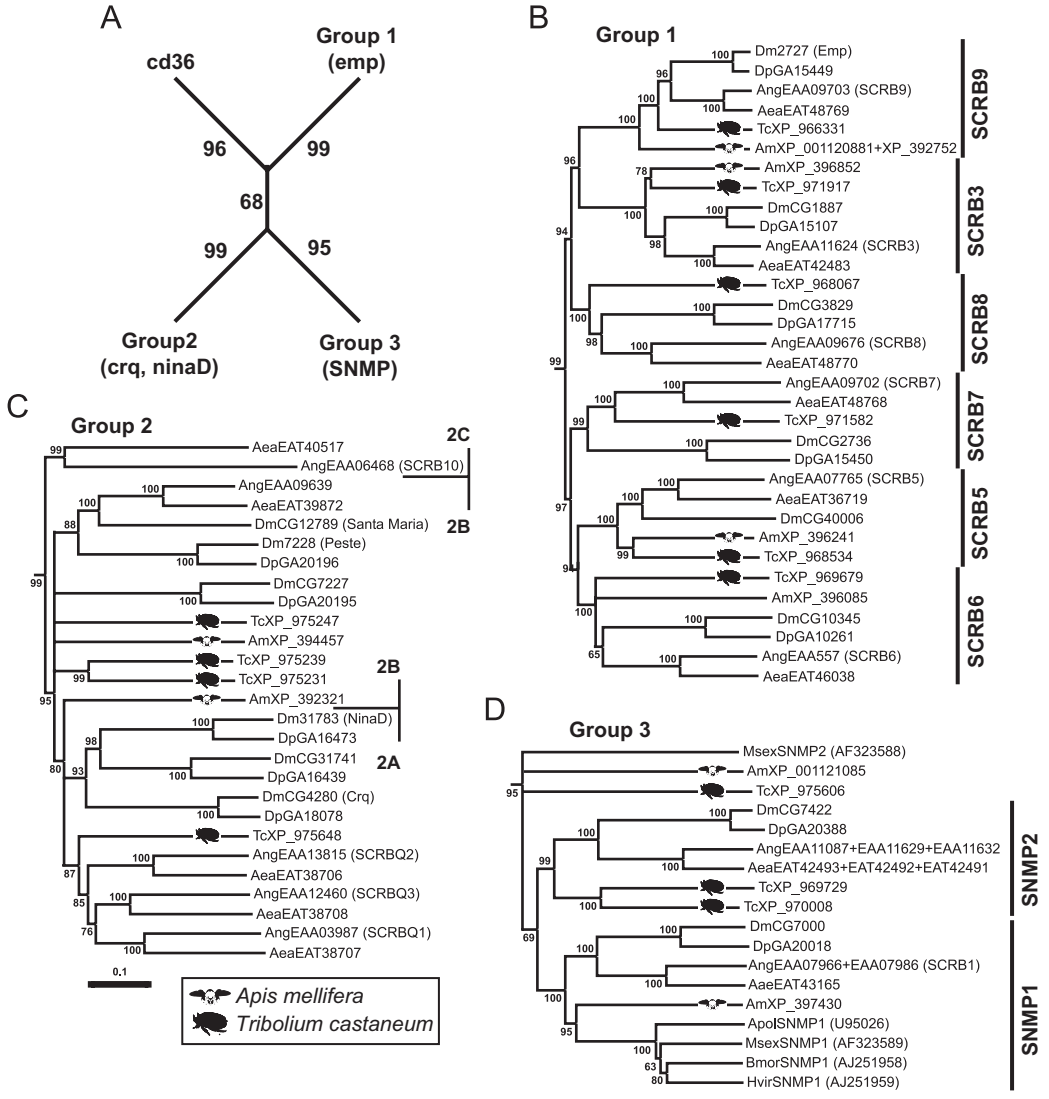

Fig. 7. Sequence comparison of Dipteran, Lepidopteran, Hymenopteran, Coleopteran, and Vertebrate SNMP/CE36 Homologs. A Neighbor Joining Tree, broken into its major components, of SNMP/CD36 homologs from *A. mellifera* and *T. castaneum* and all sequences included in Fig. 2. (A) The four major branches with bootstrap support. (B–D) Structure of individual branches (Groups 1–3 only). Subgroups indicated in Group 1 (B) are identified by the included *An. gambiae* genes (SCRB#). Subgroups indicated for Group 2 (C) are from Fig. 2A–C. *A. mellifera* and *T. castaneum* branches are emphasized by symbols. Bootstrap support is based on 1000 replicates; branches are collapsed to 50%.

subgroups with consistent representation from all four dipteran species and the coleopteran *T. castaneum*; a *D. pseudoobscura* ortholog was not identified for the SCRB5 subgroup. We identified only three Group 1 *A. mellifera* genes (Hymenoptera), suggesting a gene loss within this species or Order based on its placement between Coleoptera and Diptera. Group 2, which includes the *D. melanogaster* genes *crq*, *pes*, *ninaD*, and *santa maria*, also includes genes from all four dipteran groups as well as the coleopteran and hymenopteran species. However, the Group 2 sequences tend to group by species making the ortholgous relationships of the Group 2 genes much less clear and less conserved than those of Group 1. We identified only two Group 2 *A. mellifera* genes, compared to four to six genes for each of the other species. The Group 3 genes, which include the lepidopteran SNMPs, show a strongly supported SNMP1 and SNMP2 lineages, with one gene from *A. mellifera* associating with the SNMP1 lineage and two genes from *T. castaneum* associating with the SNMP2 lineage. *A. mellifera* and *T. castaneum* also have Group 3 genes falling outside the SNMP1 and SNMP2 lineages, suggesting additional gene duplication based complexity within this group. Overall, the general pattern of gene relationships observed for the drosophilid species (Fig. 2) appears to apply for all three holometabolous lineages and thus more than 80% of all known insect species.

The three groups do show markedly different degrees of conservation. Group 1 orthologs subgroups consistently include genes from each species, while orthologs relationships are notably less clear for the Group 2 genes. Little is known about any of the Group 1 genes; their conservation

suggests common functions across species, functions that may be central and important, and thus warrant further study. The Group 3 genes (SNMPs) also show considerable orthologs conservation. The well supported SNMP1 lineage suggests similar functions across species, a suggestion recently supported by studies of Benton et al. (2007) suggesting that *D. melanogaster* CG7000 can interact with both fly and moth pheromone receptors (see Section 4). Little is known about the SNMP2 genes; their cross species conservation again suggests a common function that warrants further study.

4.1. Overview of known CD36 homologs in mammals and insects

A bioinformatic survey identifies CD36 homologs in a broad range of phyla, including for example the Cnidaria; however, actual studies have only been carried out on members of this gene family from vertebrate and insect species. The following is a brief review of these studies. CD36 has a surprisingly broad range of described phenotypes; these phenotypes are shared by individual homologs from *D. melanogaster*. These phenotypes can be broadly simplified to include cytoadhesion, ligand transport and chemodetection.

4.1.1. Mammalian cd36 family: CD36, SCRBI, CLAI, LIMPII

CD36 is one of several related two-transmembrane domain proteins often referred to as FATs or scavenger receptors, including CD36 (Tandon et al., 1989; Oquendo et al., 1989; Greenwalt et al., 1992; Abumrad et al., 1993; Endemann et al., 1993; Calvo et al., 1998); LIMP II (“lysosomal membrane protein II”; Vega et al., 1991; Sandoval et al., 2000; Tabuchi et al., 2000); CLA-1 (“CD36 and LIMP II associated protein”; Calvo and Vega, 1993; Calvo et al., 1995), and SR-B1 (“scavenger receptor B1”; Acton et al., 1994, 1996; Rigotti et al., 1995; Krieger, 1999). CD36 was originally characterized as GPIV, a glycoprotein on platelet cells with a possible role in cytoadhesion (Tandon et al., 1989); it was noted to be immunologically related to the leukocyte differentiation antigen CD36 (e.g. Shaw, 1987) and later assumed that designation (CD stands for “cluster determinant”, referring to a cluster of antigens recognized by a specific set of antibodies directed against cell surface markers.)

CD36 has a remarkable range of reported functions, differences which may be due to alternative splice variants conferring tissue specific and/or function specific phenotypes (Andersen et al., 2006; Sato et al., 2006; Rac et al., 2007). In the broad category of cytoadhesion, human CD36 has a role in malaria, expressing in endothelial cells and binding to red blood cells infected with *Plasmodium falciparum* (e.g. Oquendo et al., 1989; Ho and White 1999; Franke-Fayard et al., 2005; Ho et al., 2005; Ayodo et al., 2007; Cunha-Rodrigues et al., 2007; Patel et al., 2007). Also in a possible cytoadhesion context and expressing in macrophage cells, CD36 and CLA-1 have roles in phagocytosis of specific bacterial species (e.g. Hoebe et al., 2005; Vishnyakova et al., 2006; Dinguirard and Yoshino, 2006; Mae et al., 2007), and CD36 has a role in phagocytosis of photoreceptor cells during outer rod segment turnover (Sun et al., 2006; Chang and Finnemann (2007).

The role of CD36 in fatty acid transport has been a major research focus, especially roles of CD36 expressed in macrophage cells as a HDL/LDL receptor and a cholesterol transporter, both in the context of atherosclerosis. Related studies have been recently and thoroughly reviewed by Febbraio and Silverstein (2007). SR-B1 also has HDL/LDL receptor and cholesterol transport activities (e.g. Acton et al., 1994, 1996; Rigotti et al., 1995; Krieger, 1999; van der Velde and Groen, 2005; Nieland et al., 2002, 2007) and is additionally reported to transport steroids (e.g. Panzenboeck et al., 2002; Langer et al., 2002) and carotenoids (e.g. During et al., 2005; During and Harrison 2007). CD36 is reported to transport fatty acids in variety of tissues, including intestine (e.g. Sukhotnik et al., 2002; Nassir et al., 2007; Levy et al., 2007) and muscle (e.g. Jeukendrup, 2002; Koonen et al., 2005; Holloway et al., 2007). Roles for CD36 have also been described in kidneys (Yang et al., 2007) and brain microglia (Abumrad et al., 2005). The brain microglial function is intriguing as CD36 has been implicated in amyloid pathways which have activities in both atherosclerosis and certain brain diseases such as Alzheimer’s (Howlett and Moore 2006; Stuart et al., 2007).

CD36 has also been implicated in taste transduction of fatty acids. Fukuwatari et al. (1997) reported the cloning of a rat FAT (later referred to as CD36 by Gilbertson et al., 2005), localizing it to a variety of tissues including the apical membranes of taste receptor cells; a role in fat detection was proposed. Coincident with this report, free fatty acids were reported to inhibit or enhance specific types of taste cell potassium channels (Gilbertson et al., 1997), and a transductory role for CD36 was further speculated (Gilbertson, 1998; Gilbertson et al., 2005). Laugerette et al. (2005) confirmed the expression of CD36 in rat taste receptor cells, and reported that CD36 null rats failed to show a normal preference for long chain fatty acids as well as a normal rise in pancreatobiliary secretions in response to oral delivery of long chain fatty acids. These studies have been followed up by several review articles suggesting that CD36 plays a significant role in taste transduction of fatty acids (Abumrad, 2005; Laugerette et al., 2006; Calder and Deckelbaum, 2006; Mizushige et al., 2007).

4.1.2. Drosophila emp (CG2727)

Emp was identified during a screen of sequences flanking a P-element insertion site in a larger study focused on wing development by Michael Wilcox (Hart and Wilcox, 1993). Emp was identified as a CD36 homolog and its temporal and spatial patterns of expression were characterized in

embryos and wing imaginal discs. Northern blot analysis indicated expression initiated in the embryo and continued into the adult stage. *In situ* hybridization analysis revealed expression was localized to ectodermal tissue beginning around 6–8 h after fertilization, primarily in embryonic epidermis and foregut/hindgut epidermis. Within wing imaginal discs, expression was observed in columnar cells which give rise to adult epidermis (wing blade and notum), persisting at least to the mid-pupa stage; expression was also observed in peripodial cells. No more work has been reported on this gene.

4.1.3. *Drosophila Croquemort (Crq) (CG4280)*

Croquemort ("maker of death") was identified in a screen looking for candidates involved in phagocytosis based on the presence of a lectin sequence motif (Franc et al., 1996). In a *crq* null mutant, macrophage cells incorporated gram positive and negative bacteria (normal) but failed to incorporate apoptotic corpses (abnormal); this abnormal phenotype was rescued using a *crq* transgene (Franc et al., 1999). Manaka et al. (2004) were unable to demonstrate failure of apoptotic uptake when Crq expression was demonstrably suppressed by dsRNA interference. However, Sears et al. (2003) did demonstrate such phenotypic failure using dsRNA interference, and further showed that Crq mediated apoptotic uptake was necessary for normal development of the central nervous system via appropriate removal of apoptosed neuronal precursors during the establishment of the neuronal scaffold. Stuart et al. (2005) also used dsRNA interference to demonstrate Crq mediated uptake of *Staphylococcus aureus* bacteria, but not *Escheria coli*; suggesting the mode of action was direct binding between the Crq protein and a cell surface molecule in the *S. aureus* membrane, coupled to the engulfment machinery of the macrophage cell via interaction between Crq C-terminus and a possible Toll-like receptor. *Crq* was recently observed to be upregulated, along with 1500 other genes, during proteomic study of viral infection in *Drosophila* cells (Go et al., 2006).

4.1.4. *Drosophila NinaD (CG31783) and Santa Maria (CG12789)*

*nina*D ("neither inactivation nor afterpotential-D") is one of eight genes (*nina*A-*nina*H) identified in a screen of chemically induced mutations affecting prolonged depolarization of photoreceptor cells (electroretinogram) following photostimulation (e.g. Pak, 1979; Johnson and Pak, 1986). The *nina* genes were interpreted and later confirmed to influence rhodopsin efficacy (Stephenson et al., 1983); *nina*E, for example, encodes opsin (Zuker et al., 1985; O'Tousa et al., 1985). The *ninaD* mutant is deficient in its ability to transport carotenoids (Stephenson et al., 1983; Giovannucci and Stephenson 1988, 1999), and thus create a functional chromophore; this transport function has been confirmed by direct biochemical analysis (e.g. Voolstra et al., 2006). The mutant gene was identified as a CD36 homolog capable of phenotypic rescue (Kiefer et al., 2002). Promoter reporter studies have indicated that *ninaD* is expressed in a broad range of larval and adult tissues, including mid-gut and brain, but notably not in the retina (Kiefer et al., 2002; Yang and O'Tousa, 2007); phenotypic rescue occurred when NinaD was ectopically expressed in non-photoreceptor neurons (Gu et al., 2004).

Santa Maria ("scavenger receptor acting in neural tissue and majority of rhodopsin is absent", or one of Columbus' ships) was recently identified as a second carotenoid transporter in *D. melanogaster* (Wang et al., 2007). Presented data suggests that NinaD is more exclusively expressed in mid-gut cells than reported in previous studies, but that a second CD36 homolog, Santa Maria, is expressed in non-retinal cells of the brain where it also functions in carotenoid transport. In a proposed scheme, NinaD would transport dietary β-carotenoid into the body in the intestine, while Santa Maria would transport circulating β-carotenoid into extra-retinal neurons and glial cells where it would be converted to all-trans retinal by NinaB (β carotene 15′ monooxigenase) and subsequently converted to all-trans retinol (vitamin A) which would then be secreted and transported to retinal cells for incorporation into opsin (Wang et al., 2007).

4.1.5. *Drosophila Peste (Pes) (CG7228)*

Peste (named after the plague) was identified in a genome wide dsRNA interference screen using *Drosophila* S2 macrophage-like cells and looking for gene products influencing uptake of and survival in the presence of *Mycobacterium fortuitum* (Philips et al., 2005). CG7228 was identified and shown to be required for uptake of *M. fortuitum*, *M. smegmatis* and *Listeria monocytogenes* but not *Escherichia coli* or *Staphylococcus aureus*. Transfected into mammalian HEK293 cells, CG7228 mediated *M. fortuitum* uptake in a manner similar to the mammalian scavenger receptors SR-BI and SR-BII. The authors suggest that CG7228 represents an evolutionarily conserved strategy in host defense, linking phagocytosis to antimicrobial signaling.

4.1.6. *Insect SNMPs*

SNMPs were first identified as the most abundant membrane protein in receptive (ciliary) membranes of sex-pheromone specific olfactory neurons of the wild silk moth *Antheraea polyphemus* (Saturniidae) (Rogers et al., 1997). This identification was prompted by the earlier characterization of a membrane protein of similar size and tissue specificity that was shown to interact with sex pheromone (Vogt et al., 1988). *A. polyphemus* SNMP1 was cloned, revealing a 59 kDa protein post-translationally processed to 69 kDa; SNMP1*Apol* was uniquely expressed in the ciliary membranes of olfactory neurons of pheromone sensitive trichoid sensilla, in the region of the cell expected to contain olfactory receptors and associating transductory proteins (Rogers et al., 1997, 2001a, b; Krieger et al., 2002). SNMP1*Apol* was suggested to have a similar two-transmembrane domain and disulfide-bond

based conformation as CD36 (Rasmussen et al., 1998). SNMPs were subsequently identified in several other lepidopteran species, including domestic silk moth *Bombyx mori* (Bombycidae), the tobacco hawkmoth *Manduca sexta* (Sphingidae) and the tobacco budworm *Heliothis virescens* (Noctuidae); two antenna specific SNMPs were identified in *M. sexta*, SNMP1 and SNMP2 (Rogers et al., 2001b). Recently, lepidopteran SNMP1 and SNMP2 were shown to differentially express in olfactory sensilla in *A. polyphemus* and *H. virescens*, with SNMP1 expressing in neurons and SNMP2 expressing in support cells (Forstner et al., 2008).

All identified Lepidoptera SNMPs express at least during the adult stage; the *M. sexta* SNMPs were shown to initiate adult expression towards the end of the pupal stage (Rogers et al., 2001a), long after antennal morphogenesis and neuronal development were complete and coincident with the expression of odorant binding proteins (OBPs) (Vogt et al., 1993) and the establishment of neuronal responsiveness to odors (Schweitzer et al., 1976). SNMPs are thought to have two-transmembrane domains, with short cytosolic N- and C-terminals and a single large and central extracellular loop (Rasmussen et al., 1998), very different in structure from the proteins that have been identified as odor receptors (ORs) (e.g. Clyne et al., 1999; Vosshall et al., 1999; Hill et al., 2002; Krieger et al., 2002, 2005; Nakagawa et al., 2005). Thus, while the spatial and temporal patterns of neuronal SNMP expression are consistent with that expected of a protein involved in odor detection, the structure of SNMPs and their relatively low diversity suggests their function is other than that of an OR.

A targeted deletion mutant of the SNMP1 gene of *D. melanogaster*, CG7000, has recently been shown to be required for detection of the aggregation pheromone *cis*-vaccenyl acetate (cVA) (Benton et al., 2007). A similar result has been obtained by Dean Smith's lab (University of Texas, personal communication). Benton and colleagues (2007) showed that, within antennae and palps, CG7000 was found to be uniquely expressed in olfactory neurons of trichoid sensilla, but also expressed in support cells of a broad range of sensilla. CG7000 was specifically required for the function of the cVA receptor (OR67d), but not for a non-pheromone receptor expressed in the OR67d neuron. CG7000 was also required for activity of a moth pheromone receptor expressed in the OR67d neuron. This study implicates SNMPs as required for pheromone detection through some SNMP-OR interaction, and further suggests a conserved mechanism for the detection and transduction of pheromone ligands, at least for Diptera and Lepidoptera, which is distinct from that for non-pheromonal ligands (Benton et al., 2007).

D. melanogaster CG7000 also expresses in chemosenory sensilla of palps, and in non-neuronal support cells of a wide variety of chemosensory sensilla (Benton et al., 2007). We have characterized CG7000 promoter driven expression of the fluorescent reporter protein cd8-GFP, observing it in a variety of chemosensory organs, including strong expression in olfactory sensilla of the antenna and palps, and what appear to be chemosensory sensilla of wings and legs (Fernandez, Dhillon, Vogt, unpublished). This expression pattern has been supported by cloned sequences from *D. melanogaster* head (accession no. ABQ96635), wing (accession no. ABQ42605) and leg (accession no. ABQ42604) (Miller, Litvack and Vogt, unpublished). This pattern of expression differs from that of the Lepidoptera SNMPs which appeared to be antenna specific (Rogers et al., 1997, 2001a) and may reflect a broader function for the dipteran vs. the lepidopteran SNMPs. The significance of this non-pheromonal and non-neuronal expression of SNMPs is unknown.

4.2. Evolutionary emergence of the insect SNMP/CD36 family

The species presented in this study represent four of the nine orders comprising the signal lineage of holometabolous insects, which undergo a complete metamorphosis from the larval to the adult stage. The holometabolous group is thought to have emerged 300+ MYA during the late Carboniferous period, and currently includes about 82% of all known insects or about 760,000 species (Grimaldi and Engel, 2005). The insect lineage in total emerged 390+ MYA (Labandeira and Sepkoski, 1993). In their recent book, Grimaldi and Engel (2005) suggest 290 MYA for the dipteran–lepidopteran/trichopteran split and 225 MYA for the lepidopteran–trichopteran lineage split. Studies based on molecular analysis suggest earlier dates (e.g. Gaunt and Miles, 2002). Earliest dipteran fossils are known from the Permian, around 281–290 MYA (e.g. Gaunt and Miles, 2002), and earliest lepidopteran fossils are known from about 190 MYA (Whalley, 1985; Grimaldi and Engel, 2005).

Within the Diptera, mosquitoes are thought to have emerged at least by the Upper Triassic period (around 215 MYA; Hennig, 1981); molecular studies suggest mosquito (*Anopheles*/*Aedes*) and fly (*Drosophila*) lineages may have split around 247–282 MYA (Gaunt and Miles, 2002). Grimaldi and Engel (2005) suggest this split occurred around 240 MYA. *Anopheles* and *Aedes* lineages may have split between 145 and 200 MYA (Krzywinski et al., 2006); *D. melanogaster* and *D. pseudoobscura* lineages may have split around 43–65 MYA (Tamura et al., 2004).

The consistent presence of orthologs SNMP/CD36 sequences in all six species argues that the overall pattern of this gene family described for Diptera in Fig. 2 was largely established at least near the base of the holometabolous lineage (300+ MYA). The structural similarity between dipteran and lepidopteran SNMPs (*D. melanogaster* CG7000, *B. mori* SNMP1) and the identification of an SNMP1 ortholog in *A. mellifera* (XP_397430) argues that the SNMP1 group was established at least within the hymenopteran/dipteran ancestor (290+ MYA). The presence of several SNMP homologs within the *T. castaneum*

argues that the SNMPs in general have their origins at least at base of the holometabolous lineage (300+ MYA; Grimaldi and Engel, 2005).

4.3. SNMP function

The mechanism by which CD36 or indeed any of these proteins work is not clear. There have been recent studies suggesting that the transport properties of CD36 involve the formation of or interaction with lipid rafts (e.g. Ehehalt et al., 2006; Atshaves et al., 2007). There have been consistent reports that CD36 interacts with cytosolic signal transduction pathways such as those involving tyrosine kinases (see review of Febbraio and Silverstein, 2007). No such information is available for the insect CD36 family members.

The insect SNMP/CD36 gene family is emerging as one of importance; described members have roles in carotenoid transport (ninaD, Santa Maria), removal of apoptotic cells and bacteria (Crq, Pes) and chemoreception (SNMP). The broad phenotypic range of CD36 may be due to alternative splice variants (e.g. Andersen et al., 2006; Sato et al., 2006; Rac et al., 2007); but no data have yet been presented concerning alternative splice variants for the insect SNMP/CD36 family members. The apparent conservation between evolutionarily broadly distant species suggests conserved protein functions between these species, at least for presumed orthologs. Almost nothing is known about the highly conserved Group 1 proteins (Fig. 2A). Several Group 2 proteins have been characterized from *D. melanogaster*, described above; each has a functional phenotype strikingly similar to one described for CD36 and its vertebrate relatives (e.g. small ligand transport or cytoadhesion). The Group 3 proteins, SNMPs, appear to be quite distinct from the others in their apparent association with chemosensory organs, and yet perhaps similar to the suggested CD36 role in vertebrate taste transduction.

We previously speculated upon several possible mechanistic roles for the lepidopteran SNMPs based on their expression patterns and apparent relationship to CD36 (Rogers et al., 1997, 2001a, b; Vogt, 2003, 2005); these roles included transport of odors and interactions with extracellular (e.g. odorant binding proteins), membrane (e.g. ORs) and cytosolic proteins. The recent work of Benton et al. (2007) suggests that SNMPs interact with pheromone receptors in olfactory neurons of the antenna, but does not address the function of SNMPs expressed in other chemosensory sensilla or in support cells. One can expect a clearer understanding of these mechanisms to emerge as the roles of the SNMPs are further studied in Diptera and other insect orders.

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